Simultaneous Densitometric Determination of Quinapril and Hydrochlorothiazide in the Combination Tablets

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Key Words:

NP HPTLC
RP TLC
Densitometry
Quinapril
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Summary

A new, simple, and accurate TLC method, using normal- and reversed-phase techniques and densitometric detection, has been developed for measurement of quinapril and hydrochlorothiazide in combination tablets. UV detection at λ = 210 nm was used to quantify the analytes. The drugs were chromatographed on silica gel 60 F_{254} HPTLC plates and on octadecilsilane (RP-18) TLC plates, in horizontal chambers, with ethyl acetate–acetone–acetic acid, 8 + 2 + 0.5 (*v*/*v*) and methanol–0.07 M phosphate buffer, pH 2.5, 6 + 4 (*v*/*v*), respectively, as mobile phases. The active substances were extracted from tablets with methanol (96% < mean recovery < 104%). Calibration curves were constructed in the range 0.4 to 2.4 µg µL^{-1} for quinapril and 0.25 to 1.5 µg µL^{-1} for hydrochlorothiazide, with good correlation ($r \geq 0.998$). The precision (*RSD* < 4.4%) and accuracy (2.91 < *RE* < 3.92) were satisfactory for TLC–densitometric determination of quinapril in combination with hydrochlorothiazide in commercial tablets.

1 Introduction

Quinapril is the esterified prodrug of the principal, active metabolite quinaprilat which blocks a specific enzyme converting angiotensin I to angiotensin II (one of the most potent vasodilators). Quinapril is widely used in hypertension and congestive heart-failure therapy. It is used in combination with hydrochlorothiazide, a diuretic agent that reduces the amount of salt and water in the body, increases the antihypertensive effects of quinapril, and is used to treat high blood pressure. Although analytical procedures – high-performance liquid chromatography [1–4], capillary electrophoresis [5, 6], and gas chromatography–mass spectrometry [7] – have been described for quantitation of quinapril in biological matrices and in pharmaceutical preparations the literature contains no report of the identification and the quantitative measurement of quinapril by TLC with densitometry. This paper reports the simultaneous determination of the two components quinapril and hydrochlorothiazide in model mixtures and tablets by both normal- and reversed-phase TLC with densitometric detection.

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2 Experimental

2.1 Chemicals

Quinapril, (3*S*)-2-[(2*S*)-2-[[(1*S*)-1-ethoxycarbonyl-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoqinolinecarboxylic acid, as its monohydrochloride, was supplied by Goedecke (Germany). Hydrochlorothiazide, 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide was obtained from Polfa (Starogard, Poland). Accuzide 20 tablets containing 20 mg quinapril and 12.5 mg hydrochlorothiazide were purchased commercially.

Methanol from Merck (Darmstadt, Germany) and ethyl acetate, acetone, and acetic, perchloric, nitric, phosphoric, sulfuric, and hydrochloric acids and *Folin–Ciocalteu* reagent from POCh (Gliwice, Poland) were of analytical grade. Potassium dihydrogen phosphate, sodium phosphate, potassium permanganate, iodine, potassium iodide, potassium iodoplatinate, ferric chloride, bismuth subnitrate, and 40% formaldehyde solution (all analytical grade) were obtained from different sources. The water used in the experiments was double distilled. The buffer was prepared by adding phosphoric acid to 0.07 M potassium dihydrogen phosphate to furnish a final pH of 2.5.

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2.2 Calibration Solutions

Stock solutions of quinapril in the range 2.0 to 12.0 mg mL^{-1} and stock solutions of hydrochlorothiazide in the range 1.25 to 7.5 mg mL^{-1} were prepared by weighing the individual compounds. To furnish quinapril–hydrochlorothiazide calibration mixtures at a constant ratio of 1.6:1 (containing 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 µg quinapril and 0.25, 0.5, 0.75, 1.0, 1.25, and 1.5 µg hydrochlorothiazide in 1.0 µL) 1.0 mL of each stock solution was pipetted into a 5.0-mL calibrated volumetric flask and the mixtures were diluted to volume with methanol.

2.3 Sample Preparation

The average mass of ten tablets containing quinapril and hydrochlorothiazide at a ratio of 1.6:1 was determined. These tablets were ground and an amount of powder equivalent to 30 mg quinapril and 18.75 mg hydrochlorothiazide was placed in a 25-mL volumetric flask containing approximately 20 mL methanol. The mixture was shaken mechanically for 30 min and then diluted to volume with the same solvent to give a solution containing 1.2 µg $µL^{-1}$ quinapril and 0.75 µg $µL^{-1}$ hydrochlorothiazide. A portion (2.0 mL) of this methanolic solution was transferred to the glass tube and centrifuged at 3500 g for 10 min. The methanolic phase was then used for chromatographic analysis.

2.4 NP HPTLC and RP TLC

Chromatography was performed on 20 cm × 10 cm HPTLC plates coated with 0.2 mm layers of silica gel $60F_{254}$ and on 20 cm × 10 cm RP TLC plates precoated with 0.25 mm layers of octadecylsilane-bonded silica gel F_{254} (Merck)

The calibration solutions (5 µL, corresponding to 2.0, 4.0, 6.0, 8.0, 1.0, or 1.2 µg quinapril and 1.25, 2.5, 3.75, 5.0, 6.25, or 7.5 µg hydrochlorothiazide) and six sample solutions (5 µL, corresponding to 6.0 µg quinapril and 3.75 µg hydrochlorothiazide) were spotted alternately as 4-mm bands (10 mm margin) on the plates by means of a Desaga AS 30 applicator equipped with a 10-µL microsyringe (Hamilton, Switzerland).

Chromatograms were developed to a distance of 50 mm in an unsaturated horizontal DS chamber (Chromdes, Lublin, Poland) with ethyl acetate–acetone–acetic acid, 8 + 2 + 0.5 (*v*/*v*) (NP HPTLC) or with methanol–0.07 M phosphate buffer, pH 2.5, 6 + 4 (*v*/*v*) (RP TLC) as mobile phases. After development the plates were dried at room temperature.

2.5 Densitometry

Densitometric evaluation was performed with a Desaga (Heidelberg, Germany) CD 60 densitometer controlled by Desaga "ProQuant" software. The chromatograms were scanned at λ = 210 nm with slit dimensions of 0.2 mm × 4 mm; λ = 350 nm was used as reference wavelength for all measurements. Concentrations of the compounds chromatographed were determined from changes in the intensity of diffusely reflected light. Evaluation was via peak areas with non-linear regression.

3 Results and Discussion

In initial assays the stability of methanolic solutions of the compounds was evaluated by UV spectrophotometric measurement of the maximum absorbance at wavelengths of 205.6 and 220.6 nm for quinapril and hydrochlorothiazide, respectively. Working standard solutions prepared at a concentration of 1 mg mL^{-1} in methanol were stable for three months at 4°C.

The application of normal-phase high-performance thin-layer chromatography (NP HPTLC) required optimization of mobile phase composition to obtain satisfactory separation of quinapril and hydrochlorothiazide. Ethyl acetate–acetone–acetic acid, 8 + 2 + 0.5 (*v*/*v*) was found to be the optimum mobile phase. Under these conditions the compounds were eluted as well shaped, symmetric spots and were completely separated with hR_F values (n = 6) of 51.0 ± 0.44 (average ± *SD*) for quinapril and 81.2 ± 0.54 (average ± *SD*) for hydrochlorothiazide (**Figure 1**). The R_S value characterizing the good separation was 2.7. The development time was 14.7 ± 0.57 min (n = 3, average ± *SD*).

Quinapril and hydrochlorothiazide on silica gel layers (HPTLC) were illuminated with UV light of l = 254 nm or visualized with a suitable chemical reagent. Details of the modes detection and the lowest amounts of compounds detected are given in **Table 1**.

The reversed-phase thin-layer chromatographic (RP TLC) retention characteristics of quinapril and hydrochlorothiazide were also studied by use of octadecylsilica stationary phase and several aqueous mobile phases containing different concentrations of methanol as organic modifier and phosphate buffer of different pH. The plot of hR_F as a function of methanol concentration [%]

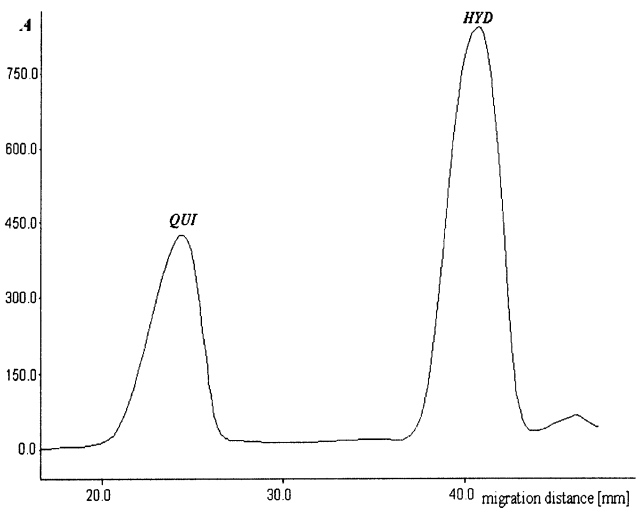

Figure 1

Densitogram (λ = 210 nm) obtained from separation of a mixture of quinapril (QUI) and hydrochlorothiazide (HYD) on a silica gel HPTLC plate developed with ethyl acetate–acetone–acetic acid, 8 + 2 + 0.5 (*v*/*v*), as mobile phase.

Table 1

Detection of quinapril and hydrochlorothiazide on HPTLC plates.

Detection reagent	Lowest detectable amount [µg] Quinapril	Hydrochlorothiazide
UV irradiation (λ = 254 nm)	5.0	0.1
Folin–Ciocalteu reagent	2.0	5.0
3% $KMnO_4$ in conc. H_2SO_4	2.5	1.0
Iodoplatinate reagent (acidified with HCl)	2.5	5.0
Iodic reagent	2.5	20.0
Dragendorff reagent (after *Amelink*)	5.0	7.5
Forrest reagent	2.0	–
Marquis reagent with Thiokol	20.0	2.5

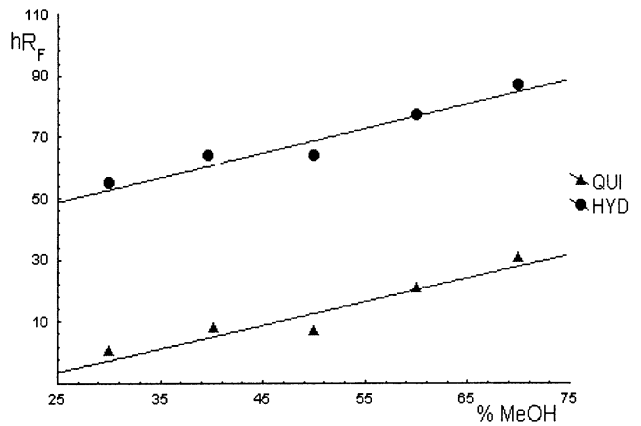

Figure 2

Plots of hR_F against concentration of methanol in the aqueous methanol mobile phase for quinapril (QUI) and hydrochlorothiazide (HYD). The stationary phase was RP-C18.

(**Figure 2**) shows that the retention of both drugs decreased when the concentration of methanol in the mobile phase was increased. The mobile phase containing 60% methanol was selected for study of the dependence of R_M on pH. The plot of R_M as a function of pH in **Figure 3** shows the relationship between the chromatographic behavior and acid–base properties of quinapril and hydrochlorothiazide. The retention of hydrochlorothiazide, a weakly acidic compound (sulfonamide group), is almost independent of pH in the range considered. The retention of quinapril increases when the pH is increased from 2.5 to 3.5 but increasing the pH further has an insignificant effect on quinapril retention. On the basis of these experiments methanol–0.07 M aqueous phosphate buffer, pH 2.5, 6 + 4 (*v*/*v*), was used for reversed-phase chromatographic determination of quinapril and hydrochlorothiazide. The drugs chromatographed as symmetric, non-tailing

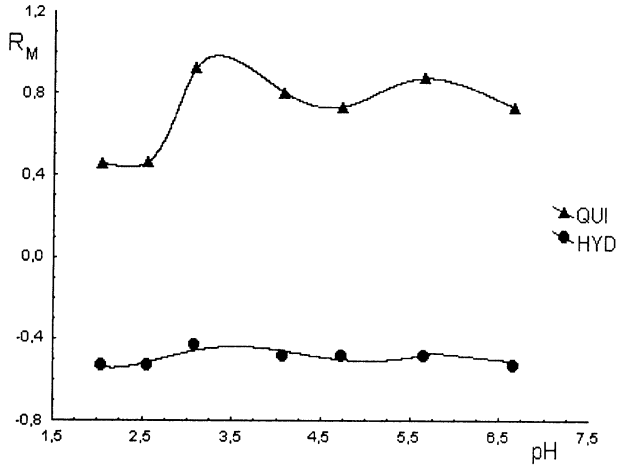

Figure 3

Relationship between the R_M values of quinapril (QUI) and hydrochlorothiazide (HYD) and the pH of the phosphate buffer in the mobile phase containing 60% methanol. The stationary phase was RP-C18.

spots with hR_F values (n = 6) of 21 ± 0.71 (average ± *SD*) for quinapril and 77.6 ± 0.56 (average ± *SD*) for hydrochlorothiazide. The R_S value calculated was 1.6. The time required for development of the plates was ca 40 min.

The principle aim of this work was the simultaneous determination of quinapril and hydrochlorothiazide by densitometry after development with the optimum NP HPTLC and RP TLC systems evaluated by means of the assays described above. To preserve the same conditions during TLC analysis calibration solutions and extracts from tablets were chromatographed on the same plate. Chromatograms obtained for quinapril and hydrochlorothiazide are illustrated in **Figures 4** and **5**.

3.1 Calibration Curves

The relationship between peak area and drug concentration was estimated in the range 0.4–2.4 µg µL^{-1} for quinapril and 0.25–1.5 µg µL^{-1} for hydrochlorothiazide (corresponding to

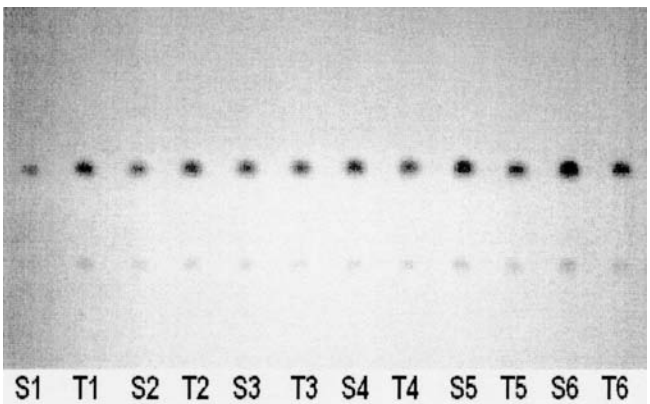

Figure 4

Chromatogram obtained from analysis of quinapril and hydrochlorothiazide calibration solutions (S1–S6) and extracts from the combination tablets (T1–T6) on a silica gel HPTLC plate developed with ethyl acetate–acetone–acetic acid, 8 + 2 + 0.5 (*v*/*v*). R_F = 0.51 for quinapril and 0.81 for hydrochlorothiazide.

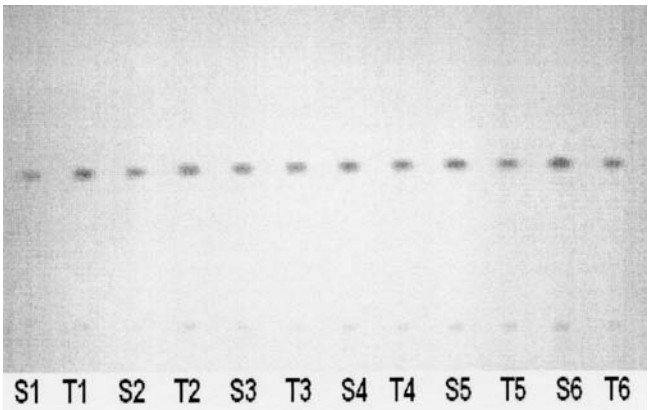

Figure 5

Chromatogram obtained from analysis of quinapril and hydrochlorothiazide calibration solutions (S1–S6) and extracts from the combination tablets (T1–T6) on RP-C18 plates developed with methanol–0.07 M phosphate buffer, pH 2.5, 6 + 4 (*v*/*v*). R_F = 0.21 for quinapril and 0.78 for hydrochlorothiazide.

2.0–12.0 and 1.25–7.5 µg/band, respectively). The calibration curves obtained were non-linear for both compounds and were expressed by means of second-order polynomial functions. The terms of the non-linear equations, with their standard errors, and the correlation coefficients obtained are given in **Table 2**.

For evaluation of repeatability three concentration levels of the analytes from the calibration range (low, medium, and high levels) were selected. Each standard solution was spotted on the same plate three times according to the application scheme: S1, S2, S3, S1, S2, S3, S1, S2, and S3. The relative standard deviations obtained (n = 3, *RSD* = 4.01–0.5%; Table 2) indicate that precision was acceptable for determination of quinapril and hydrochlorothiazide in binary standard solutions by use of these techniques.

3.2 Limit of Detection

The minimum detectable concentration, using a signal-to-noise ratio of 3 was found to be 0.38 and 0.46 µg spotted for quinapril and 0.26 and 0.36 µg spotted for hydrochlorothiazide, in NP HPTLC and RP TLC, respectively.

3.3 Accuracy, Precision and Recovery

The precision and accuracy of densitometric measurement of quinapril and hydrochlorothiazide in the tablet samples were examined as described above – n = 6, paired spotting, and calibration with samples on the same plate. The contribution of individual steps (spotting, sample preparation, chromatography) to the variability of the procedure was assessed. Two aspects of repeatability, as the precision under the same operating conditions, were considered – variability of spotting and variability of sample preparation. The first was assessed by multiple spotting of the same tablet sample (n = 6). The relative standard deviation obtained was relatively low (<2.8) for both normal- and reversed-phase chromatography (**Table 3**). The variability of sample preparation was assessed by analysis of six tablet samples prepared individually. The relative standard deviation was satisfactory (1.88–4.36) for both NP and RP methods (Table 3).

The variability of the procedure was also tested by repeated analysis of six samples on three plates within one day (in-day precision) and on three different days (day-to-day precision). The relative standard deviation expressing in-day precision varied from 2.63 to 3.50% (NP) and from 4.10 to 5.04% (RP) for quinapril and hydrochlorothiazide, respectively (**Table 4**). Similar results (*RSD*% 3.97–5.76) were obtained from evaluation of day-to-day precision (Table 4). From these results it can be concluded that the precision of RP TLC was better than that of NP HPTLC.

Tables 3 and 4 also summarize the accuracy of measurements of the drugs in tablet samples. Relative error varied from –3.23% to 3.92% for both drugs determined by use of the techniques presented. The mean extraction recovery of the two compounds from the binary tablets was in the range 96–104%. Use of *Stu-*

Table 2

Terms of non-linear regression equations $y = ax^2 + bx + c$ and results obtained from calibration analysis of quinapril and hydrochlorothiazide.

		Normal-phase		Reversed-phase	
		Quinapril	Hydrochlorothiazide	Quinapril	Hydrochlorothiazide
a (± SE)		–4.17 (± 1.08)	–28.97 (± 7.77)	– 4.84 (± 0.29)	– 9.37 (± 2.69)
b (± SE)		176.57 (± 15.40)	563.02 (± 69.42)	138.74 (± 4.20)	334.43 (± 24.09)
c (± SE)		–3.06 (± 47.08)	239.04 (± 132.64)	60.50 (± 12.84)	101.94 (± 45.02)
Correlation coefficient		0.999	0.998	0.999	0.998
Limit of detection [µg spot^{-1}]		0.38	0.26	0.46	0.36
Precision	S1	4.01	3.53	3.61	2.80
(n = 3, *RSD*%)	S3	2.09	2.98	1.92	1.20
	S6	1.10	2.17	0.85	0.50

RSD is the relative standard deviation, SE is the standard error

S1, S3, and S6 are calibration solutions affording 2.0, 6.0, 12.0 µg spot^{-1} quinapril and 1.25, 3.75, and 7.5 µg spot^{-1} hydrochlorothiazide, respectively.

Table 3

Accuracy and repeatability of spotting and of sample preparation (n = 6) for simultaneous densitometric determination of quinapril and hydrochlorothiazide in Accuzide tablets (20 mg quinapril + 12.5 mg hydrochlorothiazide/tablet).

	Spotting		Sample preparation	
	Quinapril	Hydrochlorothiazide	Quinapril	Hydrochlorothiazide
Normal-phase				
Mean amount found [mg/tablet]	19.42	12.90	20.10	12.64
Standard deviation	0.38	0.36	0.60	0.55
Standard error	0.15	0.15	0.25	0.23
Variance	0.14	0.13	0.36	0.30
Relative standard deviation [%]	1.95	2.78	2.99	4.36
95% Confidence interval	±0.40	±0.38	±0.63	±0.58
Relative error [%]	–2.91	3.23	0.48	1.16
Reversed-phase				
Mean amount found [mg/tablet]	20.36	12.75	20.03	12.79
Standard deviation	0.35	0.28	0.38	0.44
Standard error	0.16	0.12	0.15	0.18
Variance	0.12	0.08	0.14	0.19
Relative standard deviation [%]	1.73	2.22	1.88	3.40
95% Confidence interval	±0.40	±0.30	±0.40	±0.46
Relative error [%]	1.81	2.00	0.17	2.33

Table 4

In-day and day-to-day precision (n = 18) for simultaneous densitometric determination of quinapril and hydrochlorothiazide in Accuzide tablets (20 mg quinapril + 12.5 mg hydrochlorothiazide/tablet).

	In-day precision		Day-to-day precision	
	Quinapril	Hydrochlorothiazide	Quinapril	Hydrochlorothiazide
Normal-phase				
Mean amount found [mg/tablet]	19.46	12.72	19.35	12.71
Standard deviation	0.68	0.64	0.98	0.73
Standard error	0.16	0.15	0.23	0.17
Variance	0.46	0.41	0.95	0.54
Relative standard deviation [%]	3.50	5.04	5.04	5.76
95% Confidence interval	±0.34	±0.32	±0.49	±0.36
Relative error [%]	–2.72	1.76	–3.23	1.68
Reversed-phase				
Mean amount found [mg/tablet]	20.11	12.89	19.90	12.99
Standard deviation	0.53	0.53	0.79	0.71
Standard error	0.12	0.13	0.19	0.17
Variance	0.28	0.28	0.62	0.51
Relative standard deviation [%]	2.63	4.10	3.97	5.49
95% Confidence interval	±0.26	±0.27	±0.40	±0.37
Relative error [%]	0.56	3.15	–0.48	3.92

dent's t-test showed there was no significant difference between the mean recovery and 100% (95%, t calculated 0.04–0.2, which was less than the tabulated value of t).

4 Conclusion

These NP HPTLC and RP TLC densitometric methods enable simultaneous determination of quinapril and hydrochlorothiazide in combination tablets with similar correlation coefficients, precision, and accuracy. The methods presented are simple in performance, rapid, and practical and can be used as alternatives to other analytical procedures.

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